Supporting Online Material for

A B cell follicle sanctuary permits persistent productive SIV infection in elite controllers

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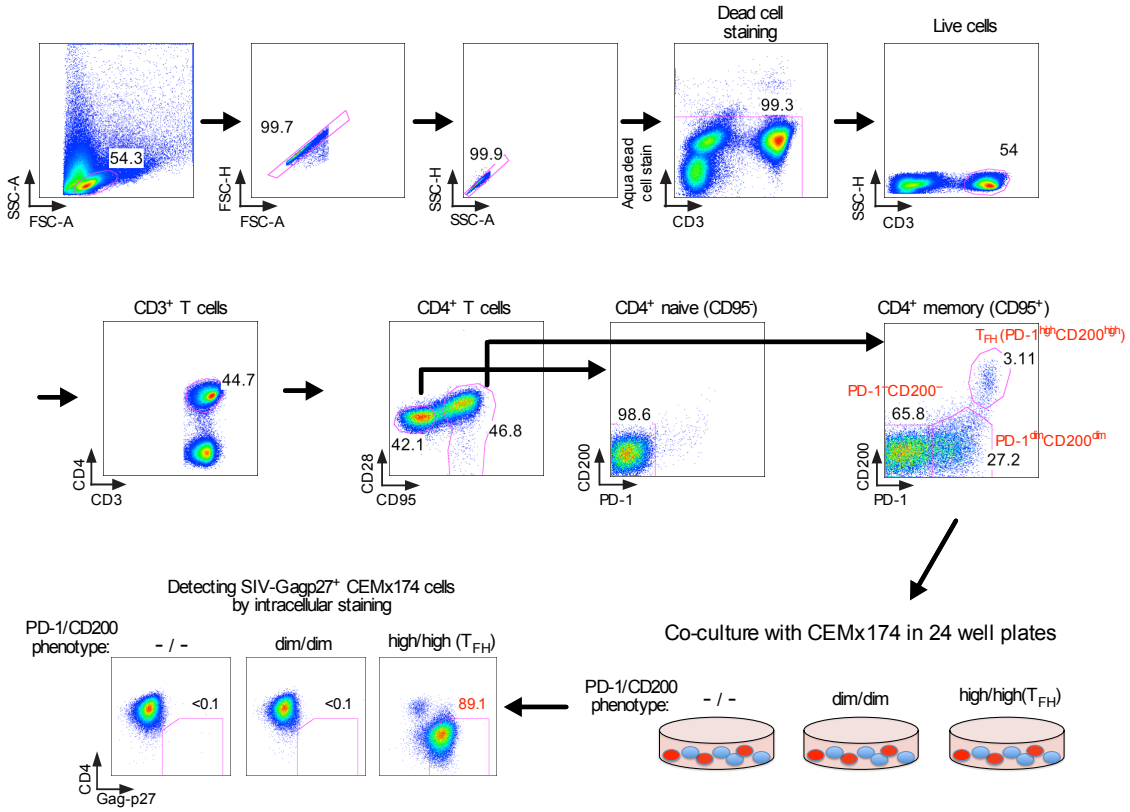
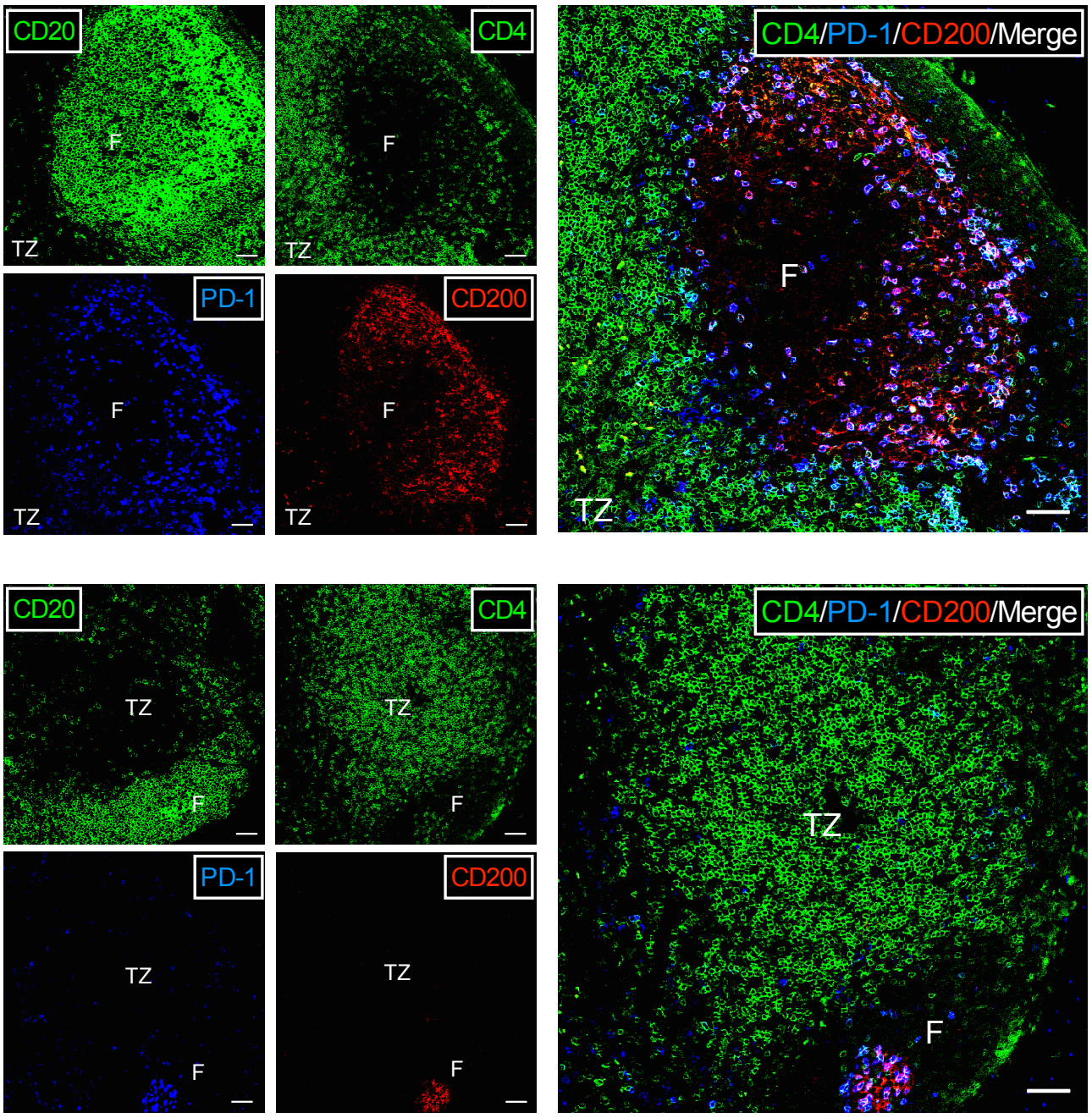
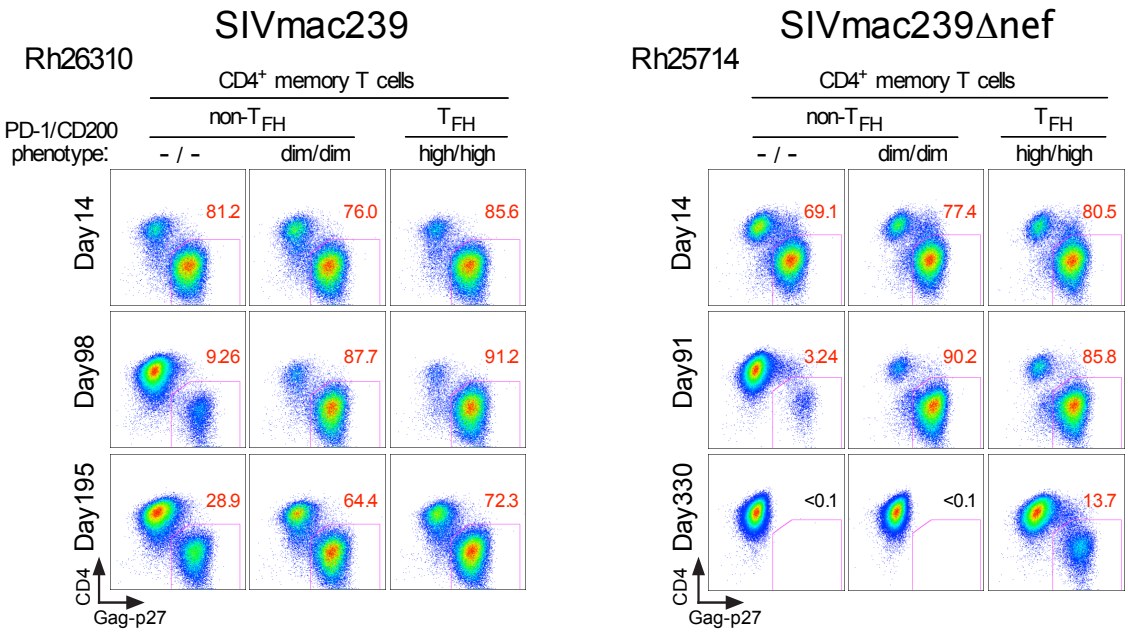
Supplemental Figures



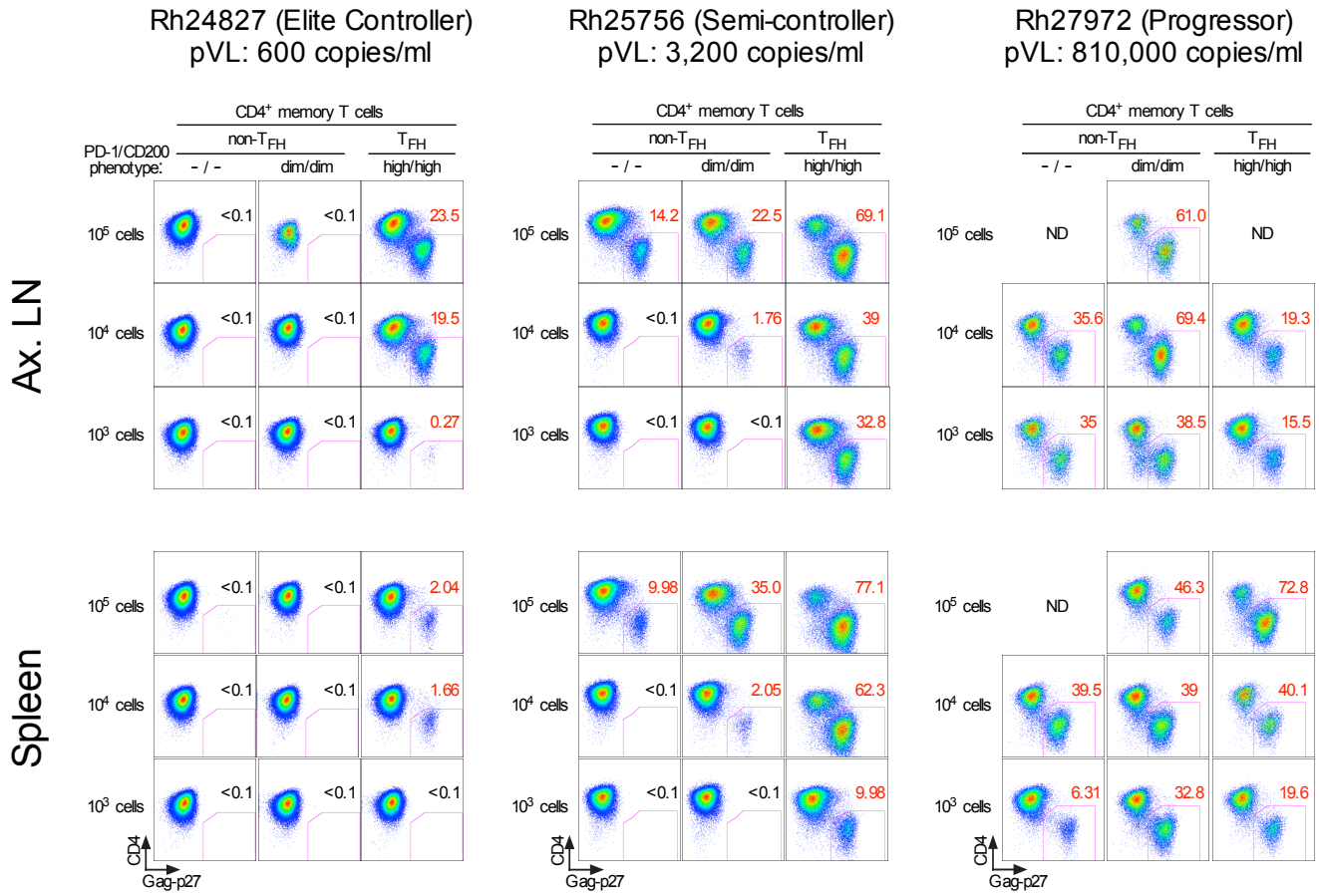
Supplemental Figure 1: Gating strategy for sorting CD4$^+$ T_{FH} and non-T_{FH} memory T cells from lymph node (or spleen) mononuclear cells. T_{FH} and non-T_{FH} CD4$^+$ memory T cells from lymph nodes were sorted as shown in the figure (elite controller monkey Rh26081). Briefly, the live, singlet, small CD4$^+$ memory T cell population (CD3$^+$CD4$^+$CD95$^+$) was sub-divided based on PD-1 and CD200 expression using the designated gates. Equivalent numbers of sorted cells (10^5 cells in this example) were co-cultured with target cells (CEMx174 cell line, 10^5 cells) for 13-36 days. Expression of SIV-Gag p27$^+$ in CEMx174 cells was detected by flow cytometry after intracellular staining cells with anti-CD3, anti-CD4, and anti-Gag p27 antibodies.



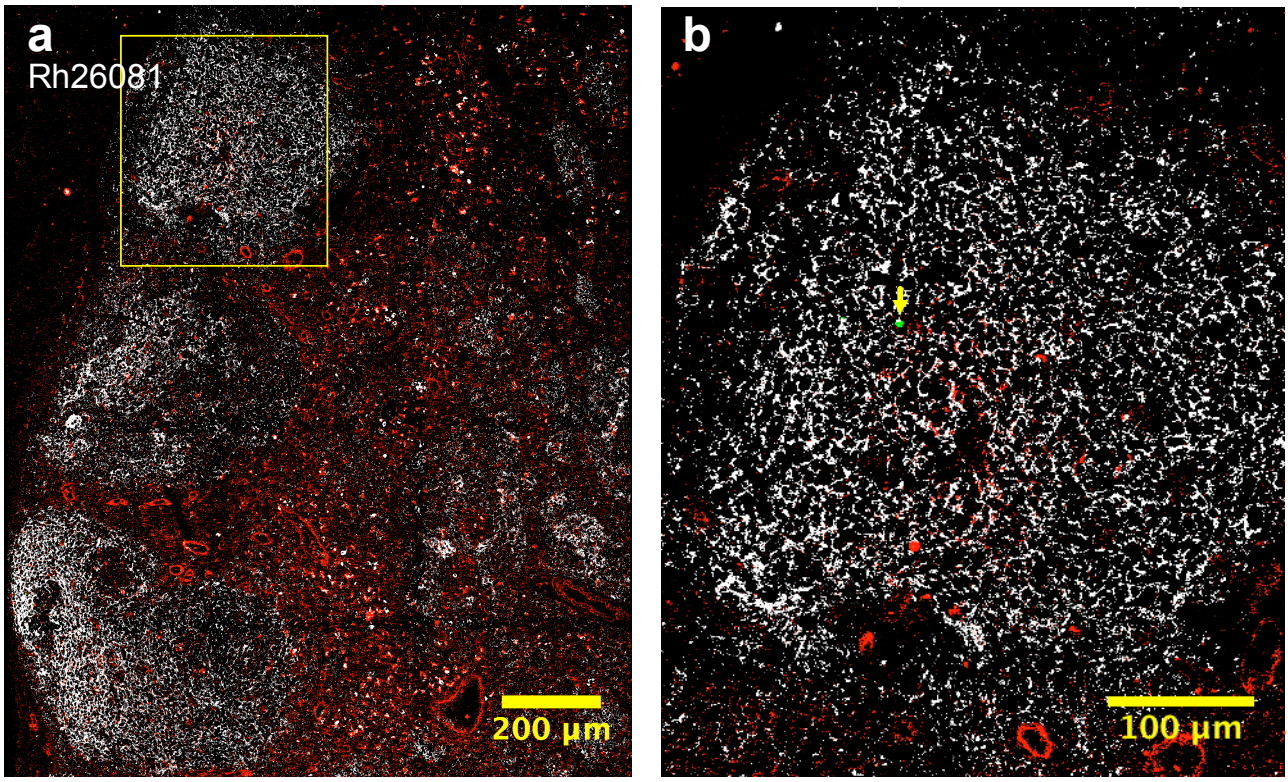
Supplemental Figure 2: Co-expression of PD-1 and CD200 specifically delineates intrafollicular CD4$^+$ T cells (T_{FH}). Triple confocal microscopy (CD4 vs. PD-1 vs. CD200) of representative lymph node sections from two SIV-infected EC monkeys (Rh24827 and Rh26623). CD4$^+$ T cells co-expressing PD1 and CD200 appear white in the merged images (far right panels; F = B cell follicle; TZ = T cell zones). CD20 confocal microscopy was performed on separate (subjacent) sections to definitively identify the B cell follicles. Note that white-colored (CD4$^+$, PD-1$^+$/CD200$^+$) cells are found only in B cell follicles (F), not in TZ. These cells are the *in situ* correlate of the CD4$^+$, PD-1^{high}/CD200^{high} T_{FH} population identified by flow cytometry. Scale bars = 50μm.



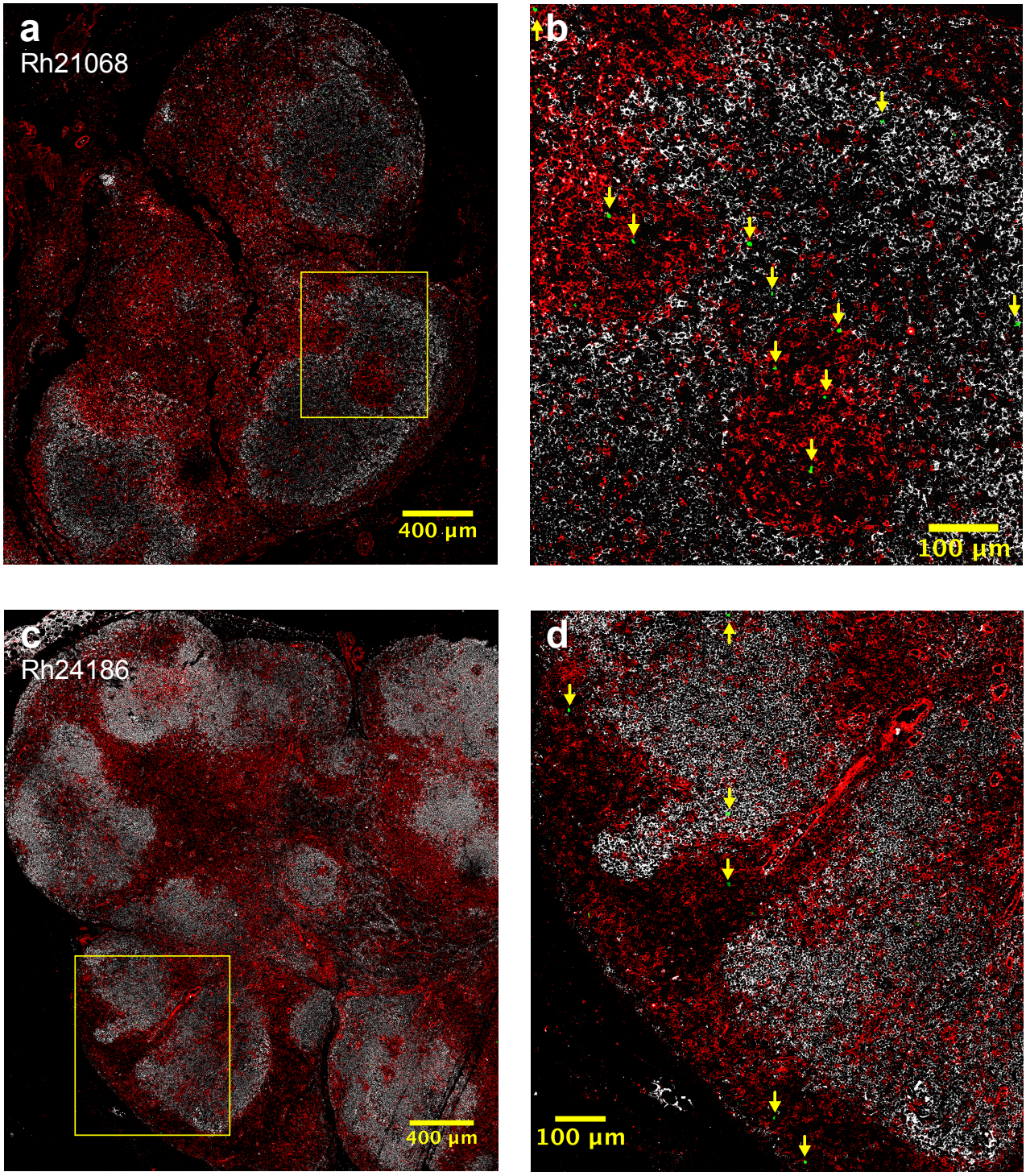
Supplemental Figure 3: Differential distribution of replication-competent SIV in lymph node cell subsets during acute and chronic infection with wildtype vs. attenuated SIV. Isolation of replication-competent virus from sorted lymph node subsets from 2 additional macaques (compare with **Fig. 1**) infected with SIVmac239 (Rh26310) vs. SIVmac239Δnef (Rh25714). 10^4 sorted lymph node cells from the designated $CD4^+$ memory T cell subsets were co-cultured for 17 days with CEMx174 cells and analyzed as shown in **Suppl. Fig. 1**. The plasma viral loads of these monkeys are shown in **Fig. 1a**. Note that restriction of replication-competent SIVmac239Δnef to $CD4^+$ T_{FH} occurred more rapidly in Rh25653 (by day 91; **Fig. 1c**) than in Rh25714 (after day 91; above) in keeping with the earlier control of viremia in Rh25653 (day 84 plasma viral load = 900 copies/ml) vs. Rh25714 (day 84 plasma viral load = 10,000 copies/ml).



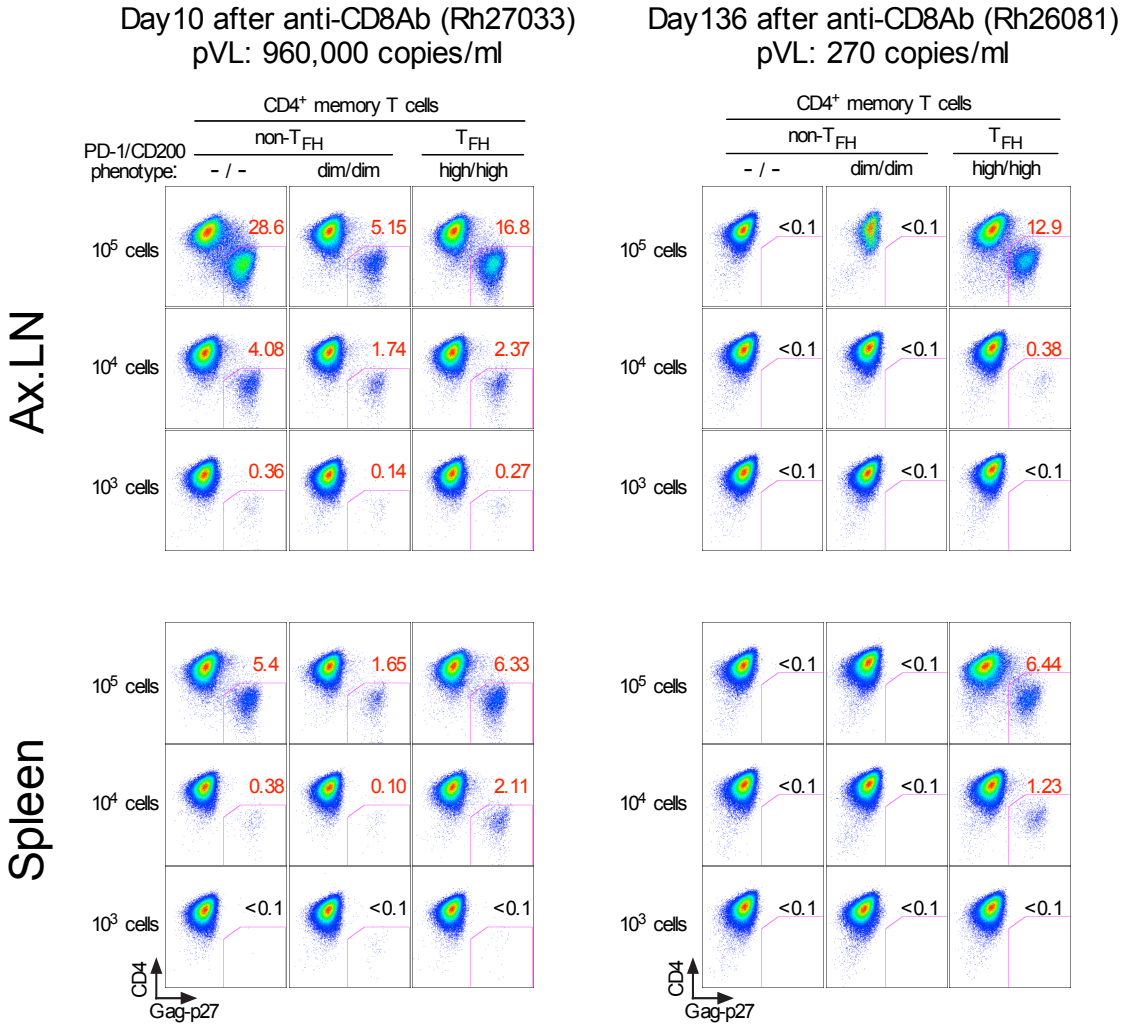
<u>Supplemental Figure 4</u>: Quantification of replication-competent SIV in CD4$^+$ T cell subsets in lymphoid tissues from chronically infected rhesus macaques. Mononuclear cells from axillary lymph nodes (Ax. LN) and spleens were sorted, and graded number of sorted cells (10^5, 10^4, and 10^3 cells) were co-cultured with CEMx174 cells for 17 days as illustrated in **Suppl. Fig. 1**. The percentage of SIV-Gag p27$^+$ in CEMx174 cells is shown for each panel. Representative data from monkeys in each designated clinical groups are shown.



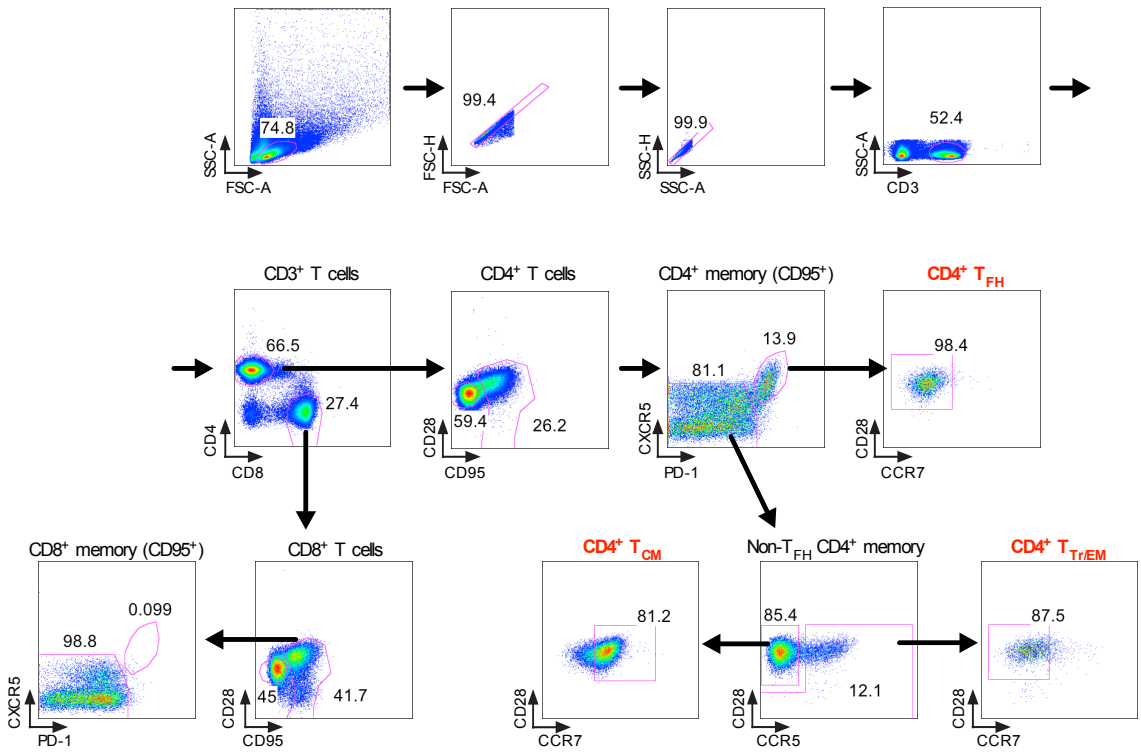
Supplemental Figure 5: *In situ* analysis of productive SIV infection in elite controller lymph nodes. The figure shows a representative confocal micrograph of a lymph node section from an additional elite controller monkey (Rh26081) with detectable SIV RNA signal. To delineate SIV RNA^+ cells, B cell follicles, and $CD8^+$ T cells in lymph nodes, the section was *in situ*-hybridized for SIV RNA (green) and double-stained with anti-CD20 (white) and anti-CD8 (red) antibodies and the image was acquired as described in Methods. **(b)** is a higher magnification image from the inset shown in **(a)**. The arrow in (**b**) indicates a productively SIV-infected cell. Note the relative paucity of CD8+ T cells (red) in B cell follicles (white).



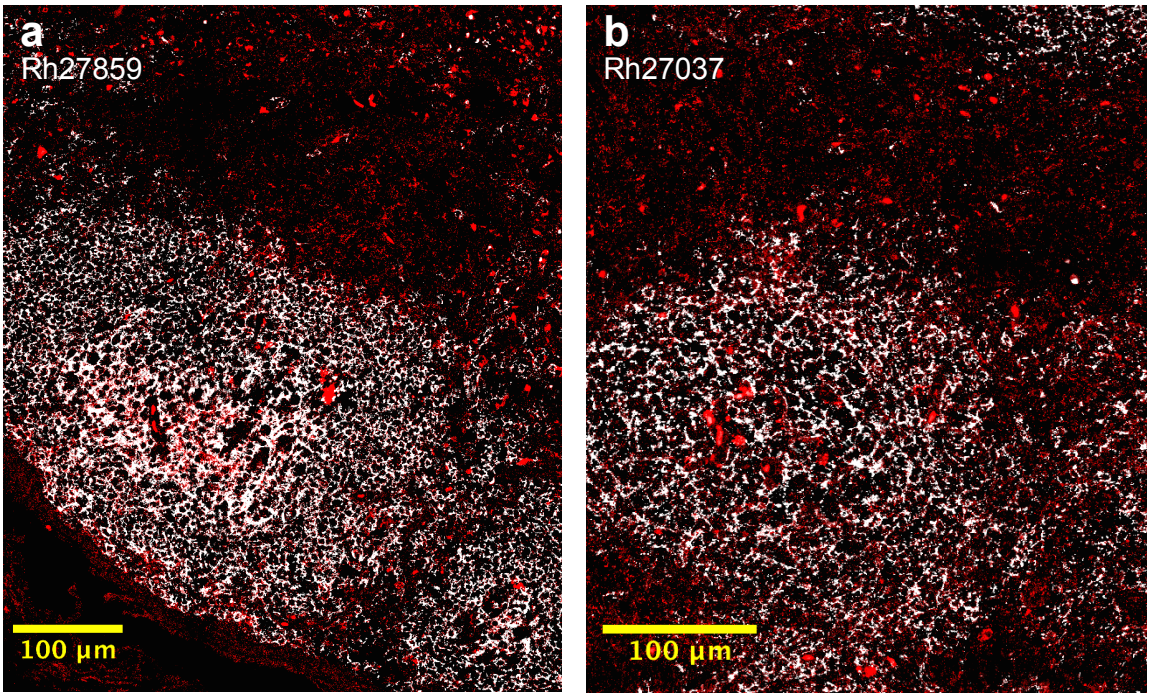
Supplemental Figure 6: ***In situ*** **analysis of productive SIV infection in lymph nodes from semi-controller and progressor monkeys.** The figure shows representative confocal micrographs of lymph node sections from semi-controller (Rh21068) and progressor (Rh24186) monkeys in which SIV RNA (green), B cells (white), and $CD8^+$ T cells (red) are simultaneously delineated (triple-stained) as described in **Suppl. Fig. 5**. (**b**) and (**d**) are higher magnification images from the insets shown in (**a**) and (**c**), respectively. The yellow arrows in (**b**) and (**d**) indicate productively SIV-infected cells.



Supplemental Figure 7: Analysis of the recovery of replication-competent SIV from CD4$^+$ T_{FH} vs. non-T_{FH} memory T cells obtained from both lymph node and spleen of EC monkeys after CD8$^+$ lymphocyte depletion. Among the 7 EC monkeys that were given anti-CD8 antibody in this study (as described in **Fig. 4**), one each was necropsied at day 10 (Rh27033) and at day 136 (Rh26081) post-CD8$^+$ lymphocyte depletion, allowing comparison of the levels of productive SIV infection in lymph node-derived vs. splenic CD4$^+$ memory T cell populations. CD4$^+$ memory T cell subsets were therefore isolated from both the axillary lymph nodes and spleens of these 2 monkeys and graded numbers of these cells (10^3, 10^4, and 10^5) were co-cultured with CEMx174 cells for 17 days and analyzed as shown in **Suppl. Fig. 1**. The percentage of SIV-Gag p27$^+$ in CEMx174 cells is shown in each profile. Note in Rh27033 that 10 days after the initial dose of anti-CD8 antibody (the peak of CD8$^+$ lymphocyte depletion), replication-competent SIV can be isolated from all CD4$^+$ memory subsets from both lymph node and spleen. Similarly, note in Rh26081 that 136 days after the initial dose of anti-CD8 antibody, at which time CD8$^+$ T cell numbers have stably reconstituted, the restriction of replication-competent SIV to CD4$^+$ T_{FH} also applies to both lymph node-derived and splenic CD4$^+$ memory T cell populations.



<u>Supplemental Figure 8</u>: Gating strategy to isolate T_{FH}, T_{CM}, and T_{TrEM} $CD4^+$ T cell subsets from lymph node mononuclear cells (used in analysis of Cohort #2 cART-treated monkeys). T_{FH} and non-T_{FH} T_{CM} and T_{TrEM} $CD4^+$ memory T cells from lymph nodes were sorted as shown in the figure (cART-treated monkey, Rh27832). Briefly, the live, singlet, small $CD4^+$ memory T cell population ($CD3^+/CD4^+/CD95^+$) was sub-divided based on PD-1 and CXCR5 expression using the designated gates. The $PD\text{-}1^{low}/CXCR5^{low}$ cells were further divided into T_{CM} ($CD28^+CCR5^-CCR7^+$) or $T_{Tr/EM}$ ($CD28^{-/+}CCR5^+CCR7^-$) based on CD28, CCR5 and CCR7 expression. Note that the $CXCR5^{high}PD\text{-}1^{high}CCR7^-$ subset is essentially identical to the $CD200^{hi}PD\text{-}1^{hi}$ subset. It is also noteworthy (lower left panels) that there are very few, if any, $CD8^+$ memory T cells with T_{FH}-like phenotype ($CXCR5^{high}/PD\text{-}1^{high}$). However, a small proportion of $CD8^+$ memory T cells (15.1 ± 1.4%, n=10) do express low-level, cell-surface CXCR5, which may endow this subset with some follicular homing capability[1].



Supplemental Figure 9: B follicular structure is intact in the RM with cART-suppressed SIV infection. The figure shows representative confocal micrographs of lymph node sections from cART-treated monkeys (Rh27859 and Rh27037; see **Suppl. Table 2**) in which $CD20^+$ B cells (white) and $CD8^+$ T cells (red) are simultaneously delineated (double-stained). Note the sharp demarcation of the B cell follicle borders, and the relative paucity of CD8+ T cells within the B cell follicles compared to the adjacent T zones, both findings indicating that follicular structure is intact in the lymphoid tissues of these virally suppressed monkeys.

Supplemental Table 1. Clinical characteristics of monkeys in designated groups

Protection	Monkey	Virus	Acute peak pVL	LN sampling day pVL	Infection days	$CD4^+$ T cells (cells/μl)	$CD4^+$ memory T cells (cells/μl)	MHC-I** (Mamu)
Elite Controller	27033*	SIVmac251	8,500,000	<30	630	1130	305	
	25327*	SIVmac251	2,900,000	40	632	1035	722	**A01**, **B08**
	25687*	SIVmac251	49,000,000	40	967	2398	1878	**B08**
pVL ≤ 600	25610*	SIVmac251	640,000	280	626	710	354	**A01**
	26623*	SIVmac239	680,000	320	277	932	359	**B17**
	23645*	SIVmac239	6,100,000	320	191	457	309	**B08**
	26372	SIVmac239	9,500,000	370	527	947	412	**A01**
	26081*	SIVmac239	3,100,000	400	315	768	619	
	21582	SIVmac239	7,200,000	510	192	1719	751	
	24827	SIVmac251	660,000	600	606	1238	937	**A01**, **B08**
Semi-controller	26136	SIVmac239	51,000,000	1,900	695	35	20	**A01**
	27591	SIVmac251	860,000	3,000	562	455	327	ND
	25756	SIVmac239	2,600,000	3,200	582	1239	586	**A01**
1,900 ≤	21068	SIVmac239	79,000	3,700	168	140	105	ND
pVL	24442	SIVmac251	12,000,000	9,500	611	455	292	
≤ 35,000	26480	SIVmac251	17,000,000	15,000	380	27	18	
	25697	SIVmac251	6,500,000	35,000	603	210	111	**A01**, **B17**
Progressor	24955	SIVmac251	12,000,000	85,000	563	104	89	**A01**
	19262	SIVmac239	2,100,000	120,000	277	225	185	**A01**, **B17**
85,000 ≤ pVL	27705	SIVmac239	81,000,000	270,000	434	126	49	
	29412	SIVmac239	85,000,000	270,000	337	339	34	
	26515	SIVmac251	21,000,000	340,000	337	911	77	
	P735	SIVmac251	127,000,000	520,000	498	98	75	
	P383	SIVmac251	97,000,000	710,000	602	241	104	
	F55	SIVmac239	2,600,000	710,000	91	273	68	
	27972	SIVmac239	49,000,000	810,000	439	448	113	
	24186	SIVmac239	19,000,000	920,000	233	597	380	**B08**
	P436	SIVmac251	12,000,000	1,700,000	566	208	85	
	26310	SIVmac239	110,000,000	12,000,000	195	445	43	
	27617	SIVmac239	11,000,000	16,000,000	274	1760	43	
	26303	SIVmac239	44,000,000	19,000,000	337	239	150	

*: monkeys used for the CD8 depletion experiment; **: MHC-I shows known protective haplotype (A01, B08, or B17);
pVL: plasma SIV RNA copies/ml; ND: not determined

Supplemental Table 2. Plasma viral loads (pVL) of monkeys that received combination antiretroviral therapy (cART)

Cohort #1

Monkey	Virus	Infection days	cART days	Acute peak pVL	Pre-ART pVL	Post-ART pVL
24269	SIVmac251	427	155	7,900,000	170,000	<30
25708	SIVmac251	413	162	3,300,000	45,000	<30
25960	SIVmac251	378	169	150,000,000	19,000	40
26460	SIVmac251	407	126	18,000,000	31,000	60
26550	SIVmac251	422	141	900,000	170,000	60
26576	SIVmac251	505	224	23,000,000	4,800	<30
26687	SIVmac251	427	148	30,000,000	150,000	<30

Cohort #2

Monkey	Virus	Infection days	cART days	Acute peak pVL	Pre-ART pVL	Post-ART pVL
27037	SIVmac239	223	181	13,000,000	2,800,000	50
27832	SIVmac239	223	181	8,300,000	1,100,000	<30
27835	SIVmac239	223	181	5,700,000	470,000	<30
27857	SIVmac239	223	181	12,000,000	670,000	<30
27859	SIVmac239	223	181	19,000,000	790,000	<30
27913	SIVmac239	223	181	5,400,000	350,000	<30
27919	SIVmac239	224	182	81,000,000	1,300,000	50
28157	SIVmac239	224	182	11,000,000	180,000	<30
28337	SIVmac239	224	182	7,600,000	360,000	<30
28763	SIVmac239	224	182	12,000,000	2,800,000	50

pVL: plasma SIV RNA copies/ml